PHENOLIC GLYCOSIDES FROM *PINUS SYLVESTRIS* L

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SYNOPSIS

The presence of phenolic glycosides in the needles of *Pinus sylvestris* L has been studied. By a combination of chromatography on Sephadex LH-20, anion-exchange resin and silicic acid, a series of phenolic glycosides have been isolated. Besides the 3′-glucosides of quercetin and 2,3-dihydro-quercetin (taxifolin) previously isolated from other conifers, several isomeric glucosides of guaiacylglycerol and p-hydroxyphenylglycerol, and various glycosides having aglycones of dilignol type also have been isolated. The latter glycosides are: a glucoside and a rhamnoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol; a glucoside, a rhamnoside, and a xyloside of 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-hydroxyphenoxy]-1,3-propanediol; and two glucosides and an arabinoside of (+)-isolariciresinol. The phenolic glycosides are also present in other parts of the tree.

The presence of some nonphenolic extractives is briefly discussed.

INTRODUCTION

Before discussing a series of phenolic glycosides which we have isolated from the needles of *Pinus silvestris* L—the most common pine in Scandinavia—it is advisable to sum up some other low-molecular extractives which have been previously isolated from the same source [1,2]. Among the hydrophilic extractives (water-soluble part of an acetone extract), mainly carbohydrates and the related cyclitols, some components such as glucose, fructose, sucrose, pinitol, and shikimic acid were isolated in a yield of 1.5–2.5% each. The fact that the three former sugars together constitute around 7% of the dry weight (although there are seasonal and individual variations) is a notable energy contribution where the needles are intended for use as animal feed. In the next group of compounds present in the range of 0.1–1%, L-arabinose, L-rhamnose, D-mannitol, melibiose, raffinose, myoinositol, and sequoitol were isolated, and, laminaribiose and cellobiose were found, for the first time, as free disaccharides among minor low-molecular carbohydrate constituents.

In the lipophilic part of the acetone extract (soluble in methylene chloride) a diterpene acid, named pinifolic acid (Fig. 1), was shown to be the main acid constituent (1–2% of dry weight) [3]. Dehydropinifolic acid, which has a C(13)–C(14) double

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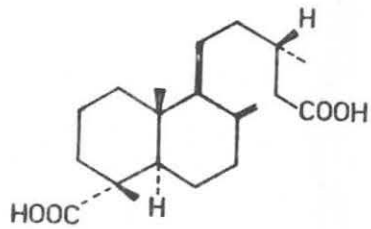

FIG. 1. Pinifolic acid.

bond, as well as benzoic acid, among the other acid components were later identified [4]. We demonstrated that neither pinifolic acid nor dehydropinifolic acid were present in other parts of the same tree; the two acids were not detected in the needles or in the oleoresins of Norway spruce (*Picea abies*) or European larch (*Larix decidua*).

RESULTS AND DISCUSSION

Isolation of Phenolic Glycosides

Carbon-Celite chromatogràphy (eluant: aqueous ethanol) was used for the fractionation of the low-molecular carbohydrates [1,2]. In connection with the isolation of the various carbohydrate constituents, two glucosides, giving positive color reactions with phenol reagents, were also isolated (in about 0.1% yield each) [2]. Both were identified as β-D-glucopyranosides of guaiacylglycerol, the sugar not being linked to the phenolic group, as in coniferin, but to aliphatic hydroxyls in the glycerol side chain. One was crystalline ($[\alpha]_D + 45°$), the other amorphous ($[\alpha]_D - 16°$). Periodate treatment indicated that glucose was linked in the α-position in the former and in the β-position in the latter glucoside. The aglucones from both glucosides were shown to be threo-isomers. In later studies at the Swedish Forest Products Research Laboratory, using fractionation on Sephadex LH-20 columns and elution with water and aqueous ethanol, we also found indications of the presence of the γ-isomer and at least two of the corresponding glucosides of p-hydroxyphenylglycerol [5]. (These types of glycosides were eluted as group 3; Fig. 2.) The anion-exchange resin Dowex 1 (in acetate form), used in connection with removal of acidic constituents from the water-soluble part of the acetone extract, provided a somewhat unexpected tool for fractionating these types of glycosides. They were eluted after the saccharides and well before the more high-molecular glycosides (group 4; Fig. 2) and partially fractionated from each other.

We are working, currently, on the isolation of these monocyclic glycosides for further identification by a combined fractionation on ion-exchange and silicic acid columns. These compounds, which are also present in other parts of the tree, are of interest in connection with the discussion of the biosynthesis of lignin and other wood constituents having phenylpropane structures.

We often enrich the phenolic glycosides and other hydrophilic phenolic compounds from the main carbohydrate constituents by extraction with 2-butanone. The yields given in Figure 2 are from such an extract from an autumn collection (there may be quite considerable seasonal and individual variations). This first chromatographic step usually results crude fractionation, but sometimes pure compounds may be ob-

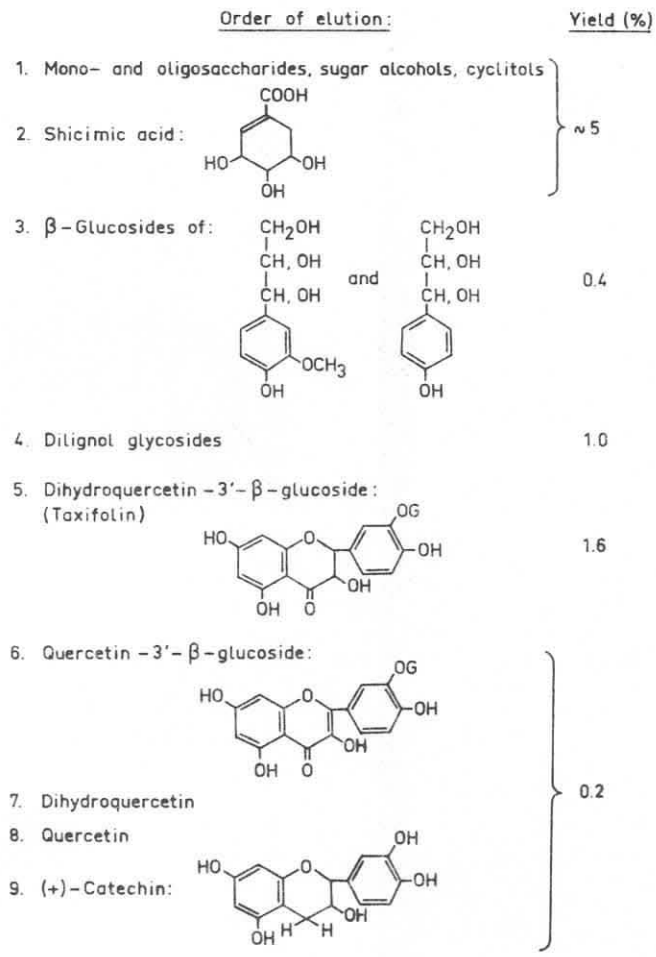

FIG. 2. Fractionation of n-butanone soluble (and water-soluble) extractives from pine needles (autumn) on Sephadex LH-20 (eluent: water and aqueous ethanol).

tained in one run. The normal procedure for obtaining pure compounds from the complex mixture is to continue with chromatographic subfractionations on silicic acid columns, preparative thin-layer chromatography, or high-pressure liquid chromatography.

After the group of monocyclic glycosides (of guaiacyl- and p-hydroxyphenylglycerol), discussed above, we obtained a complex mixture (group 4; sometimes in a higher yield than in this example) which turned out to be dilignol glycosides—which we fractionated and identified. Dihydroquercetin (taxifolin)-3′-β-glucoside, eluted after that group, is the major glycoside. After that compound, smaller amounts of the related quercetin-3′-β-glucoside and the corresponding aglycones are eluted. These four compounds have been previously isolated from many conifers by Goldschmidt and Hergert [6]. Finally, the well-known (+)-catechin and related compounds are eluted.

The dilignol glycosides were separated and obtained in a pure state after repeated fractionation, and identified as glycosides of the three aglycones shown in Figure 3. Figure 4 is an idealized schematic illustration of their chromatographic properties.

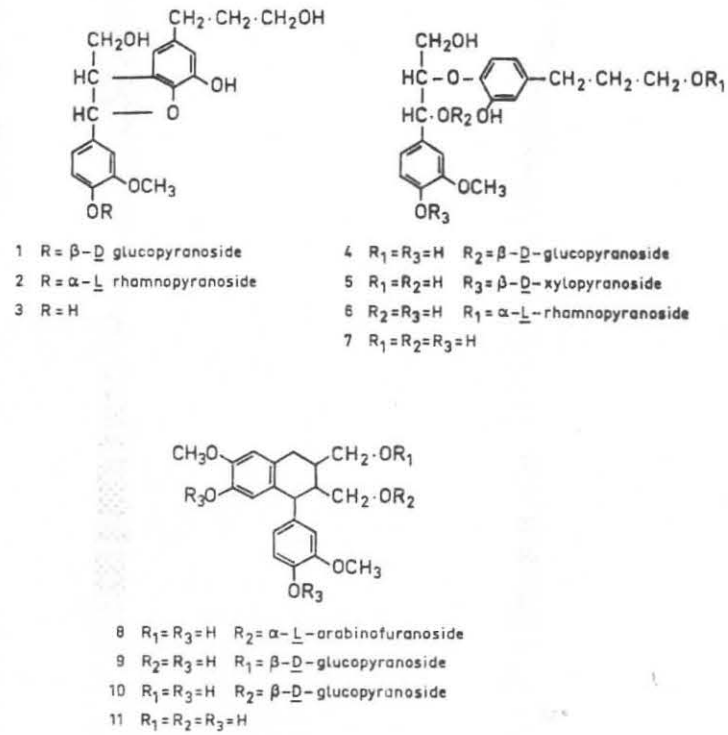

FIG. 3. Dilignol glycosides and aglycones.

on which the purification procedure is based. A hexoside of a certain aglycone (such as the glucoside 4) is eluted from the Sephadex LH-20 column before the corresponding pentoside (such as the xyloside 5), and the latter before the 6-deoxy-hexoside (as the rhamnoside 6) (Fig. 4). In a similar way, glucoside 1 is eluted before rhamnoside 2, and glucosides 9 and 10 before arabinoside 8. In TLC chromatography on

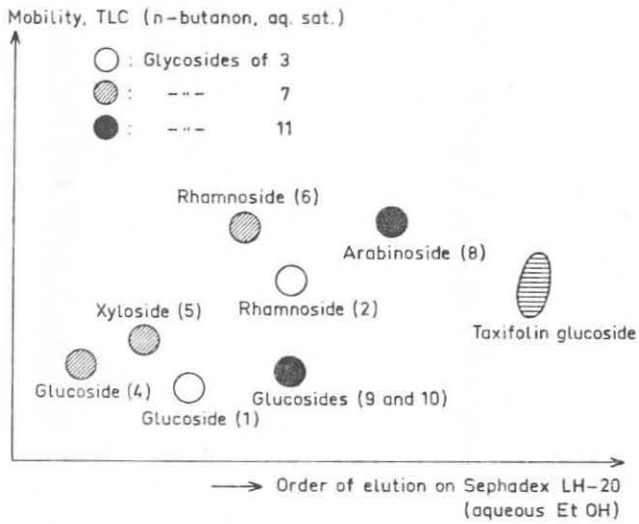

FIG. 4. Fractionation of dilignol glycosides from pine needles.

silicic acid, on the other hand, rhamnoside 6 has a higher mobility than has glucoside 4. Arabinoside 8, being furanosidic, also exhibits characteristically high mobility compared with pyranosidic xyloside 5.

Identification of the Eight Dilignol Glycosides

In addition to NMR and MS for the identification in general, methylation and studies of the products after subsequent hydrolysis are also important for showing the position of the glycosidic linkage to an aglycone, and for proving whether the sugar part is pyranosidic or furanosidic. (A report about the identification of compounds 1–3 will appear in the near future [7], and a more complete account of the isolation and identification of the eight glycosides and the corresponding aglycones will be published elsewhere.) Aglycone 3 in glycosides 1 and 2 has not been previously re-

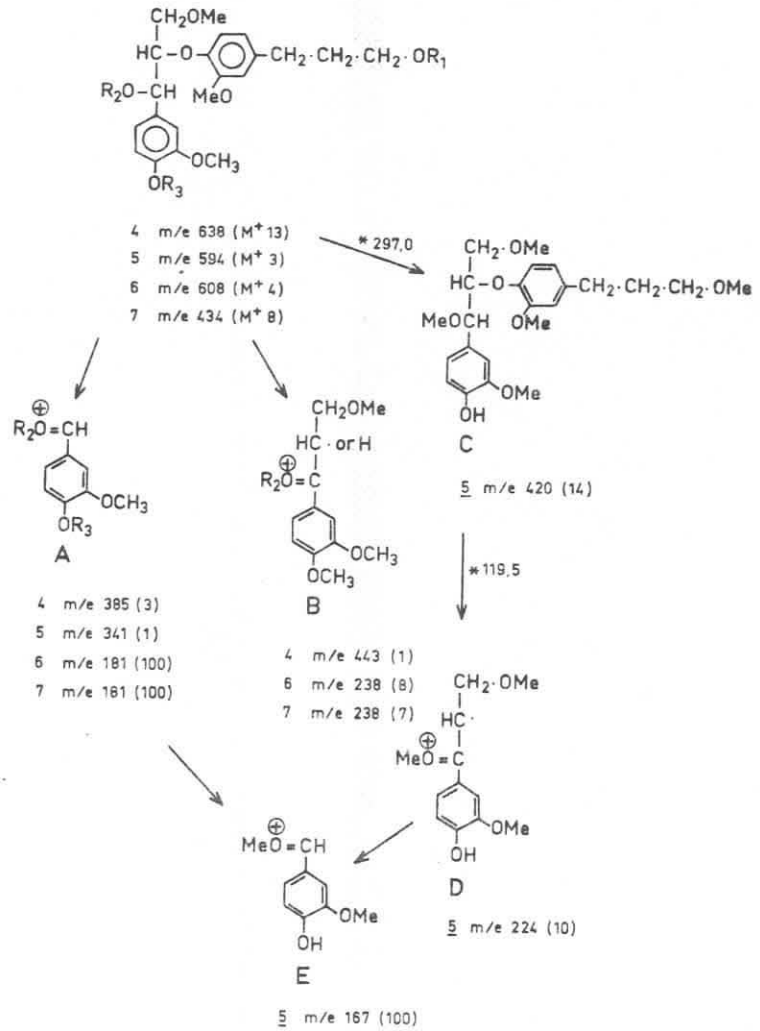

FIG. 5. Mass spectrometric fragments from methylated compounds 4–7.

ported. It was isolated in a crystalline state (mp 137–139°C: $[\alpha]_D$ + 5.4°C) after acid hydrolysis of the glucoside 1 (present in ca. 0.2% of the dry weight of the needles) or rhamnoside 2 (ca. 0.4% yield). The sugars were isolated and identified. Elemental analysis and MS of the aglycone, showing a strong peak corresponding to the molecular ion, confirmed the suggested structure. Further, by methylation of either of the glycosides, followed by acid hydrolysis, dihydrodehydrodiconiferyl alcohol—identical with an authentic sample of the well-known model compound for phenylcoumaran structures in lignin—was obtained. We also obtained peaks in MS, corresponding to the expected molecular ions of the fully methylated glycosides. Aglycone 3 was present also in the free state in the extract. The saturated state of the side chain and the fact that one of the aromatic rings is pyrocatecholic is a notable fact both in this and the next group of dilignol compounds (4–7) to be discussed.

Glycosides 4, 5, 6 as well as aglycone 7, which was also isolated from the extract, showed very similar NMR-spectra, indicating the presence of six aromatic protons, one benzylic proton (doublet around δ 4.9), one methoxyl group, and six protons characteristic for the propanol side chain, among others. Acidic hydrolysis of 4, 5, and 6 decomposed the aglycone but the sugars could be identified. We could, however, hydrolyze compounds 4 and 5 but not rhamnoside 6 enzymatically to the aglycone 7. The rhamnoside, however, was found to be identical with a glycoside—previously isolated by Manners and Swan [8] from *Thuja plicata*.

NMR of the acetylated compound 4 showed, contrary to acetylated 5 and 6, no downfield shift for the α-proton, proving that the glucose in compound 4 is linked to the benzylic alcohol. The NMR of acetylated compound 5, on the other hand, showed only one aromatic acetyl, proving that xylose is linked to a phenolic hydroxyl group. We thus have here the interesting and unique situation of three different sugars linked to three different kinds of hydroxyl groups in the same aglycone. We have looked very carefully for the other combinations but have not found any; if they are present, they must only be traces. Results from the mass spectrometric studies of this group of compounds are shown in Figure 5.

After methylation, glycosides 4–6 and aglycone 7 all gave peaks corresponding to the expected molecular ions. Fragment A is very significant. The occurrence of peaks 385 and 341, and the absence of peak 181 for compounds 4 and 5, respectively, proved the presence of a hexose-tetra-O-methyl and a pentose-tri-O-methyl residue in fragment A from 4 and 5, respectively. Notice also that only compound 5 (having the xylose in a phenolic position) showed the occurrence of fragments C, D, and E, while compounds 4, 6, and 7 gave fragment B. Aglycone 7, obtained from compound 4 by enzymatic hydrolysis, has a threo-configuration in the glycerol chain, as was previously found in the guaiacylglycerol glycoside [2], while the aglycone from compound 5 is a mixture of threo- and erythro-isomers in the ratio 1:3. Compound 6 has the threo-configuration.

The third group of dilignol glycosides have (+)-isolariciresinol (11) as an aglycone, which was readily shown after hydrolysis. Methylation studies proved that the sugar units are linked at either of the aliphatic hydroxyl groups. One glucoside of lignan 11 has been reported previously [9].

We have shown the presence of the dilignol glycosides, but in smaller amounts, in other parts of the tree. At present, we are also studying the changes in the composition of carbohydrates, resin acids, and phenolic compounds when the needles turn brown on the tree and when present in the litter after various times. There are still some low-molecular carbohydrates and phenolic glycosides left after six months in the

ground but in much decreased amounts. The aim of the work in progress is to see also how various extractives influence the growth of various lignin- and carbohydrate-degrading microorganisms in the forest ground.

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